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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:
Shuji SUMIDA et al.
Serial No. 09/622,487
Filing Date: August 17, 2000
For: PROTEIN-FREE FORMULATION

Examiner: RUSSELL, Jeffery E.
Group Art Unit:1654

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner of Patents and Trademarks,
Washington, D.C. 20231

Sir,

I, Yasushi SATO, a Japanese citizen, c/o Chugai Seiyaku Kabushiki Kaisha, 5-1, Ukima 5-chome, Kita-ku, Tokyo 115-8543, Japan, hereby declare that I am one of the inventors of the above-entitled patent application and that I received a B.Sc. degree from Tohoku University, Faculty of Science in March 1988 and a M.Sc degree from Tohoku University, Graduate School of Science in March 1990.

I declare also that I have been employed by Chugai Seiyaku Kabushiki Kaisha, the assignee of this application, and have been engaged in formulation research since April 1990 and that I work as a supervisor of Pharmaceutical Technology Division of Chugai Seiyaku Kabushiki Kaisha.

I declare further that I have read all of the Office Actions in the above-entitled patent application, and have read and am familiar with each of the references cited in the Office Actions cited by the Examiner.

I declare further that I am familiar with the subject matter disclosed in said

application as well as the disclosures in the references cited against the claims, including Tsuji et al. (U.S. Patent No. 5,202,117), Michaelis et al. (U.S. Patent No. 5,919,757) and JP No. 4-77436A.

I declare that the following statements are true and correct to the best of my knowledge.

Statements

The following experiments were conducted in order to demonstrate that the stability of G-CSF-containing formulations is significantly improved at pH 6.8 or lower pH as compared to stability at pH 7.0.

Experiments

1. Method

The same source of G-CSF was used for all of the experiments.

Mixtures of G-CSF, polysorbate 20 or polysorbate 80, and sodium chloride as an isotonic agent were prepared and adjusted to various pHs, as shown in the following Table 1, with a sodium phosphate buffer.

Table 1

Lot No.	G-CSF	Surfactant		pH	Isotonic agent
U3L01	100 micro g/mL	0.1mg/mL	polysorbate 20	pH 7.0	Sodium Chloride
U3L02	100 micro g/mL	0.1mg/mL	polysorbate 20	pH 6.8	Sodium Chloride
U3L03	100 micro g/mL	0.1mg/mL	polysorbate 20	pH 6.6	Sodium Chloride
U3L04	100 micro g/mL	0.1mg/mL	polysorbate 20	pH 6.5	Sodium Chloride
U3L05	100 micro g/mL	0.1mg/mL	polysorbate 80	pH 7.0	Sodium Chloride
U3L06	100 micro g/mL	0.1mg/mL	polysorbate 80	pH 6.8	Sodium Chloride
U3L07	100 micro g/mL	0.1mg/mL	polysorbate 80	pH 6.6	Sodium Chloride
U3L08	100 micro g/mL	0.1mg/mL	polysorbate 80	pH 6.5	Sodium Chloride

Each formulated solution was prepared under sterile conditions and filtrated, after which amounts of 0.5ml were filled into grass vials and sealed to prepare a G-CSF liquid formulation.

The thus prepared formulation containing 100 micro gram / ml of G-CSF was then allowed to stand in an incubator at 40°C for 2 weeks.

The content of G-CSF was determined according to the following method 1 as described on page 11 of the present specification.

Method 1

Pure water, acetonitrile and trifluoroacetic acid were used as mobile phase on a C4 reverse phase column (4.6 mm x 250 mm, 300 angstroms). The content of G-CSF was determined by reverse phase high-performance liquid chromatography. The amount equivalent to 5 μg of G-CSF was injected and G-CSF was eluted with an acetonitrile gradient and spectroscopically detected at a wavelength of 215 nm.

The G-CSF content determined by this method was used to calculate the remaining percentage (%) after acceleration at 40°C for 2 weeks according to the following equation.

Remaining percentage (%) = [(G-CSF content after acceleration at 40°C for 2 weeks) / (G-CSF content without acceleration)] x 100

2. Results

The results are shown in the following Table 2.

Table 2

Lot No.	Surfactant	pH	Remaining percentage	
			Average	Standard deviation
U3L01	polysorbate 20	pH 7.0	85.2	1.6
U3L02		pH 6.8	90.4 **	1.1
U3L03		pH 6.6	94.0 **	1.1
U3L04		pH 6.5	92.9 **	0.6

Mean and standard deviation values are calculated for seven samples.

** : significantly different ($p<0.01$) for data of U3L01

Lot No.	Surfactant	pH	Remaining percentage	
			Average	Standard deviation
U3L05	polysirbate 80	pH 7.0	63.8	0.3
U3L06		pH 6.8	83.5 **	1.5
U3L07		pH 6.6	86.5 **	0.9
U3L08		pH 6.5	90.6 **	1.6

Mean and standard deviation values are calculated for seven samples.

** : significantly different ($p<0.01$) for data of U3L05

3. Conclusion

A pH value has a strong influence on a remaining percentage of G-CSF in G-CSF-containing formulations. In the present experiments, it was shown that there was a significant difference between U3L01 (pH7.0 , polysorbate 20) and U3L02 (pH6.8, polysorbate 20) determined by the t-test assay on the level of 0.01. It was also shown that there was a significant difference between U3L05 (pH7.0,

polysorbate 80) and U3L06 (pH6.8 , polysorbate 80) . It is concluded that stability of G-CSF-containing formulations is significantly improved at pH 6.8 or a lower pH as compared to that at pH 7.0.

I declare further that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Dated this second day of February, 2004

Yasushi Sato

Yasushi SATO